# MOUSE IMMUNOGLOBULIN QUANTIFICATION KIT

FastELISA®

Catalog Number RDB 3256 (96 tests)

## APPLICATION

The mouse immunoglobulin quantification kit provides a rapid and easy method **(one step ELISA)** for the quantitative determination of mouse IgG in cell culture supernatant and ascitic fluid.
The kit includes ready-to-use reagents necessary to analyze up to **90 samples** in **30 min. Buffer solutions are color coded in order to simplify pipetting steps.**

## PRINCIPLE OF THE ASSAY

The method employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific to mouse IgG (H+L) is pre-coated onto microwells. Samples and standards are pipetted into microwells and mouse IgG present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-mouse IgG (H+L) antibody is pipetted and incubated simultaneously with samples. After washing microwells in order to remove any non specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of mouse IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

## SPECIFICITY

The method enables the detection of all IgG (IgG3 quantification requires a specific standard curve).
Cross reactions (determined by ELISA) are < 1% for Human IgG, < 1.5% for Cow IgG, < 2% for Goat IgG, < 5 % for Swine IgG and < 15 % for Guinea Pig and Rat IgG.
The cross reaction with human serum and fetal calf serum is typically below 0.2%.

## SENSITIVITY

The detection range is from **20 ng/ml to 1900 ng/ml**.
The detection threshold is **6 ng/ml**.

## STORAGE

All kit components are stable for 12 months when stored at 2-8°C. Do not freeze.
After opening, reagents must be handled with care to avoid contamination and should be used within 2 months.

## KIT CONTENTS

| Code | Component | Quantity |
|---|---|---|
| RDB3256-P | Pre-coated microplates: 96 microwells coated with anti-mouse IgG (H+L) polyclonal antibodies | 6 strips of 16 wells |
| RDB3256-Sd | Mouse IgG standards (Blue solution) Concentrations: 0 – 0.02 – 0.1 – 0.2 – 0.75 – 1.9 µg/ml | 6 x 0.3 ml |
| RDB3256-D | Sample Diluent (PBS pH7.4, 1% BSA, 0.1% Tween 20) (Blue solution) | 30 ml |
| RDB3256-C | Detection antibody: Peroxidase conjugated anti-mouse IgG (H+L) polyclonal antibody (Red solution) | 12 ml |
| RDB3256-T | Substrate solution (TMB) | 12 ml |
| RDB3256-St | Stop solution (2M HCl) | 12 ml |

*All the kit components are ready-to-use*

## ADDITIONAL MATERIAL REQUIRED

- Pipettes and tips (20-200 µl).
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at **450 nm** and 620 nm.
- Wash solution: $H_2O$, 0.05% Tween 20. Other wash solutions may be used but they have to be tested with the method.

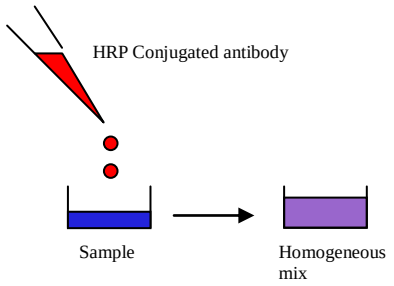
## SAMPLE PREPARATION AND STORAGE

If necessary, samples may be stored at -20°C prior to perfrom the assay. Dilute the samples in the sample diluent (Blue). Recommended dilution factor are indicated in the following table:

| Samples | Recommended dilutions |
|---|---|
| Cell culture supernatant | 1/100 |
| Miniperm, CELLine supernatant | 1/1000 |
| Ascitic fluid | 1/10 000 |

## ASSAY PROCEDURE

All steps must be performed at room temperature (RT). Bring all reagents at RT for 30 min before use.

| | |
|---|---|
| STEP 1 | Perform the dilution of each sample in diluent buffer. Serial dilutions may be necessary as recommended previously. |
| STEP 2 | Add 20 µl of samples or standards per microwell. |
| STEP 3 |  Pipette without delay in the same order 100 µl of peroxidase conjugated anti-mouse IgG (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the plate for **15 min** at RT. |
| STEP 4 | After incubation, remove the solution and wash the plate three times each with **300 µl** of the wash solution. An automatic plate washer is recommended. |
| STEP 5 | Pipette 100 µl of TMB substrate into each well. Incubate the plate for **10 min** at RT. |
| STEP 6 | Stop the reaction by pipetting 100 µl of STOP solution in the same order as for TMB distribution. |
| STEP 7 | Read the absorbance at **450 nm** and 620 nm with a microplate reader. |

## CALCULATION OF RESULTS

**Validation of the assay:** The mean absorbance of the 0 ng/ml standard should be below 0.1 AU (absorbance unit). Maximal absorbance (1900 ng/ml standard) should be around 1.6 to 2.2 AU, depending of the operating temperature.

**Standard curve:** plot the average value (**absorbance 450**-620) of each standard on the Y axis against their corresponding concentration on the X axis. Software able to generate a cubic spline curve-fit is recommended.
The mouse IgG concentration in the sample can be calculated by interpolation between standard points on the curve.
**Note:** It is recommended to repeat the assay at a different dilution factor in the following cases:

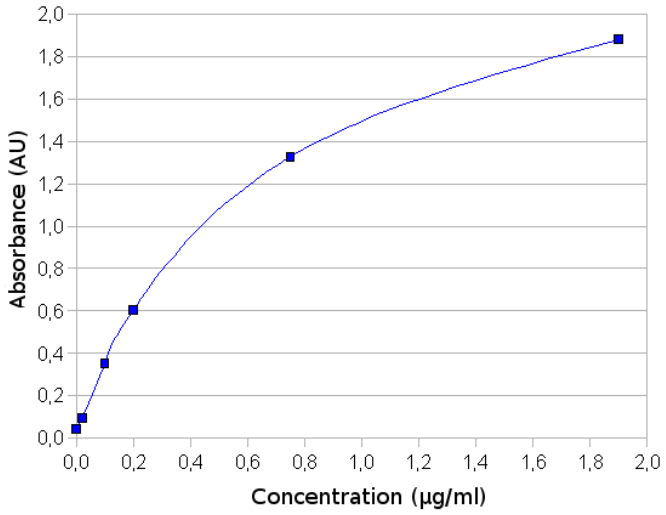
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 1900 ng/ml standard.

**Hook effect:** a hook effect may be observed at IgG concentrations above 5000 ng/ml. Serial dilution of the sample is then recommended.

FOR RESEARCH USE ONLY
RD-Biotech – 3, rue Henri Baigue – 25000 Besançon – France – Phone. +33 3 81 53 88 37 – Fax. +33 3 81 53 89 65
email: rd-biotech@rd-biotech.com - www.rd-biotech.com

## TYPICAL DATA

This standard curve is shown as an example only. A new standard curve should be performed for each series of samples to be tested.




## PRECISION

Intra-assay precision

| Sample | Dilution | Mean concentration (µg/ml) | SD (%) | Number of measures |
|---|---|---|---|---|
| Supernatant A | 1/100 | 10.63 | 5.33 | 9 |
| Supernatant B | 1/100 | 11.56 | 7.47 | 9 |
| Supernatant C | 1/100 | 22.61 | 8.48 | 9 |
| Supernatant D | 1/100 | 28.88 | 10.03 | 9 |
| Supernatant E | 1/100 | 66.82 | 8.39 | 9 |
| Supernatant F | 1/100 | 75.92 | 9.9 | 9 |
| Supernatant G | 1/100 | 102.47 | 10.38 | 9 |

Inter-assay precision

| Sample | Dilution | SD (%) | Number of measures |
|---|---|---|---|
| Supernatant H | 1/250 | 3.45 | 30 |
| Supernatant H | 1/500 | 2.99 | 30 |
| Supernatant H | 1/1000 | 4.97 | 30 |